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# THE ESCAPE OF VELIGERS FROM THE EGG CAPSULES OF *NASSARIUS OBSOLETUS* AND *NASSARIUS TRIVITTATUS* (GASTROPODA, PROSOBRANCHIA)[1]

JAN A. PECHENIK

*Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543 and Massachusetts Institution of Technology, Cambridge, Massachusetts 02139*

Many species of prosobranch gastropods deposit their eggs in tough capsules affixed to hard substrates. Generally, there is a small opening near the top of such capsules, occluded by a firm plug (operculum) which must be removed before the veligers can escape. The sizeable oothecan literature deals primarily with basic descriptions—size, shape, number of eggs or embryos contained, where and when the capsules are found in the field (*e.g.*, Anderson, 1966; Bandel, 1974; D'Asaro, 1969, 1970a, 1970b; Franc, 1941; Golikov, 1961; Graham, 1941; Knudsen, 1950; Kohn, 1961; Ponder, 1973; Radwin and Chamberlin, 1973; Thorson, 1946). The remaining studies deal mostly with the structure and chemical composition of the capsules (*e.g.*, Bayne, 1968; Fretter, 1941; Hunt, 1966), rather than with how the young escape.

In a review paper on the hatching of aquatic invertebrates, Davis (1968, p. 336) suggested that the removal of the plug is usually attributable to embryonic secretion of enzymes. However, most of the ideas about how this first step in the hatching process is accomplished are without experimental support, deriving solely from descriptions of the process (*e.g.*, Bandel, 1974; Chess and Rosenthal, 1971; Davis, 1967; Houbrick, 1974; Kohn, 1961; Murray and Goldsmith, 1963; Portmann, 1955). The limited experiments which have been reported (Ankel, 1937; De Mahieu, Perchaszadeh, and Casal, 1974; Hancock, 1956; Kostitzine, 1940), deal exclusively with species that emerge from their capsules as crawling, juvenile snails. These experiments suggest that a hatching substance is produced by advanced embryos, but no attempt was made to determine the nature or properties of the substance, or the timing of its production.

The belief in a chemically-mediated release of young is not universal. West (1973, p. 4) has suggested that the capsule plug of *Colus stimpsoni* is degraded by "external factors such as bacteria and fungi"; this idea lacks any experimental support.

The egg capsules of *Nassarius obsoletus* and *N. trivittatus* are quite similar in size (approximately 1.5 mm high), number of eggs contained (forty to several hundred), and general morphology (Scheltema and Scheltema, 1964); and both have openings occluded by plugs of approximately 100 $\mu$ in thickness. Hatching takes place at the veliger stage for both species, after about one week of encapsulated development (Scheltema and Scheltema, 1964; Scheltema, 1967).

This paper demonstrates that a specific hatching substance is produced by encapsulated embryos of both species and examines some of its properties and

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the timing of its production, primarily for *N. obsoletus,* through laboratory experimentation.

In addition, the actual escape of veligers from the egg capsules of *N. obsoletus* was examined. Due to the large size of the egg capsule relative to the size of an individual veliger, the small size of the opening of the capsule, and the jelly-like consistency of the intracapsular fluid (Costello and Henley, 1971), one might expect that many hours would be required for the capsules to become empty if the veligers were swimming about aimlessly. One to two days are required for all of the embryos to leave the capsules of the freshwater gastropod *Acroluxus lacustris* (Gamulin, 1973). However, if movements within the egg capsule were directed towards the opening of the capsule, much less time would be required.

I use the terminology of Giese and Pearse (1974). "Embyo" refers to an individual which has not yet taken up a free-living existence, whereas the term "larva" refers to a free-swimming individual which has entered the plankton to complete its development.

## Methods

Egg capsules were obtained from snails held in the laboratory. Specimens of *Nassarius obsoletus* were collected from the mudflats at Barnstable Harbor, Massachusetts, and specimens of *N. trivittatus* were collected by dredging in Buzzard's Bay, Massachusetts. All animals were fed on shredded clam meat.

The experiments fall into two main categories—those designed to examine the production of a hatching substance by embryos and its functional longevity, and those designed to detect its continued production by hatched larvae. Shelled, pigmented embryos were obtained from intact capsules by slicing into the capsule wall with a razor blade and expelling the embryos with gentle squeezing of the capsule using forceps. Other capsules were monitored hourly until hatching occurred; larvae from these capsules were then used at known times from the onset of hatching.

To assay for the production of hatching substance, a known number of embryos or larvae were first pipetted into the bottom of a small glass chamber, whose empty weight had been determined on a Roller-Smith Precision balance. The chamber was then reweighed and the weight difference before and after filling used to estimate the volume of water in the chamber. A freshly-deposited egg capsule was then sliced in half, parallel to its long axis, so that each half contained one part of the plug firmly held in the sectioned neck (Fig. 1). One half of this capsule was placed at the bottom of the chamber with the animals, while the other half of the same capsule was placed in a similar chamber with sea water but no embryos or larvae, as a control. Chambers were examined daily for three to six days, or until the plug was observed missing from the neck of the capsule. Chambers were held in air-tight containers at room temperature (20–23° C), at a relative humidity of 100%. Embryos and larvae remained active under these conditions for at least six days. Five-micron filtered sea water was used in all experiments.

The functional longevity of the hatching substance was assessed by placing intact egg capsules into individual glass chambers in a small volume of water (approximately 10 $\mu$l) and monitoring until hatching began. The actual volume

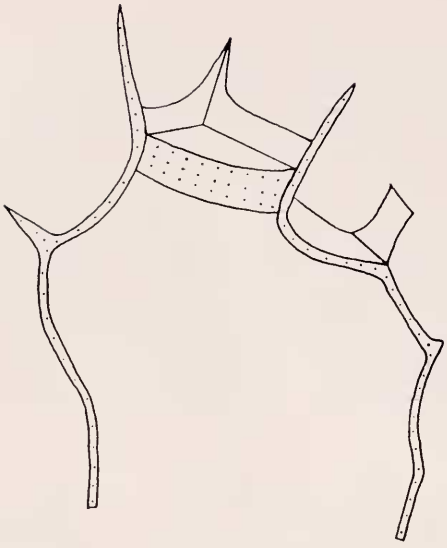

FIGURE 1. Diagramatic illustration of a *Nassarius obsoletus* egg capsule which has been sliced parallel to its long axis to expose the plug in the mouth of the capsule (drawn from a photograph). The base of the capsule has been cut away. Cut surfaces are stippled.

of water in each chamber was determined by weighing, as above. One hour after hatching had begun, the chamber fluid was removed to a new glass chamber. Fresh plug material was then added to the hatching fluid from 0 to 3 to hours later, and its integrity examined several days later. The other halves of these capsule opercula were held in sea water as controls.

Specific details for each experiment are given in the tables. In experiments testing the species specificity of hatching substance, plugs of both species were included with the advanced embryos of one species; the control plug was produced by the species whose embryos were being tested.

Additional controls were set up to examine the possibility of eventual spontaneous plug detachment. Capsules containing osmotically-killed eggs and embryos were held for two months in five-micron filtered sea water at room temperature; periodically these were gently squeezed to assess plug integrity. Freshly deposited egg capsules were emptied of their contents and sliced in half as described above, and their opercula examined after two months in sea water at room temperature.

To compare experimentally-determined densities of embryos required to initiate plug release with the actual density of embryos in egg capsules, the volumes of four *N. obsoletus* capsules which had hatched out known numbers of larvae were estimated with a microsyringe.

An equation predicting the rate of collisions of confined gas molecules with the walls of their container was used to predict the rate at which veligers would leave an egg capsule if their escape were a purely chance process. The equation is (Sears, 1959): number of collisons per unit area per unit time $= \frac{1}{4}$ n $\bar{v}$, where "n" is the number of molecules per unit volume, and "$\bar{v}$" is the average speed of

the molecules in the container. The equation assumes that the gas molecules are uniformly distributed in the container. By treating the encapsulated veligers as gas molecules and assuming that the collison of a veliger with the opening at the top of the capsule results in the escape of the individual from the capsule, the rate at which veligers should leave is predicted by multiplying the above expression by the surface area of the opening of the egg capsule, 0.08 $mm^2$ ($N = 2$). The average speed of encapsulated veligers was determined by analyzing movie footage of the movements inside an emptying egg capsule when only 8 veligers remained, by which time individual movements could be followed. The model egg capsule was assumed to initially contain 50 veligers. The value of "n" in the equation was changed after each hypothetical 5-minute interval by subtracting the number of escapees in the interval from the number present at the beginning of the interval and dividing by the hypothetical egg capsule's volume, 0.6 $\mu$l. Predicted rates of escape were compared with those actually observed in the laboratory.

## Results

From the time the capsule is deposited until just prior to hatching, the capsule plugs of both species have very distinct outlines and are firmly held in the necks of the egg capsules (Fig. 1). The plugs do not actually dissolve in the hatching process, but become amorphous, greatly softened and are easily dislodged from the capsule wall. Plug degradation seems to be a sudden event, rather than a gradual process.

No control plugs became softened or loose in the necks of their egg capsules over the two-month observation period or in the course of any particular experiment. Those plugs destined to be degraded generally fell away from the necks of the sliced egg capsules by the day following the start of the experiment. This supports the contention that degradation of the plug does not involve a gradual build-up of hatching substance within the capsule.

The results of 14 experiments in which different numbers of embryos were confined in volumes of sea water ranging from 2.4 $\mu$l to 230.0 $\mu$l are presented in order of increasing embryonic density (Table I). Plug release was scored in all chambers holding more than 0.4 embryos per $\mu$l, regardless of the absolute number of individuals contained.

Although no clear cut-off time is revealed, the secretion of hatching substance is no longer detectable four hours after hatching for *N. obsoletus*, and by 24 hours after hatching for *N. trivittatus* (Table II). Indeed, production by *N. obsoletus* larvae seems to decline within one hour of hatching, since plugs were degraded only at the highest densities of one-hour old larvae. Note that all larval densities used in these experiments were well above those previously found adequate for plug dissolution by embryos (Table I). The hatching substance is thus no ordinary metabolite, but rather a substance produced specifically to loosen the capsule plug prior to escape of the veligers. Larvae which have apparently ceased production of hatching substance do not degrade capsule opercula, indicating that physical manipulation does not play a major role in removing the plug.

TABLE I

*Production of hatching substance by advanced embryos of* Nassarius obsoletus. *Plus represents release of plug from capsule neck; minus represents plug remaining firm in capsule neck.*

| Number embryos | Number embryos per μl | Outcome |
|---|---|---|
| 45 | 0.196 | − |
| 55 | 0.239 | − |
| 2 | 0.400 | − |
| 4 | 0.408 | + |
| 210 | 0.913 | + |
| 6 | 1.000 | + |
| 16 | 1.176 | + |
| 45 | 1.364 | + |
| 16 | 1.454 | + |
| 329 | 1.645 | + |
| 16 | 1.951 | + |
| 13 | 2.241 | + |
| 13 | 2.500 | + |
| 16 | 4.473 | + |

The hatching substance produced by *N. obsoletus* embryos loses its potency within 3 hours in sea water at room temperature (Table III).

Despite the similarity between the capsules of the two species examined, the substance produced by the embryos of one species is incapable of degrading the plugs of the other (Table IV).

TABLE II

*Production of hatching substance by larvae of* N. obsoletus *and* N. trivittatus (*) *at various times after the initiation of hatching. Plus represents release of plug from capsule neck; minus represents plug remaining firm in capsule neck.*

| Hours after initiation of hatching | Number larvae | Number larvae per μl | Outcome |
|---|---|---|---|
| 1 | 14 | 2.641 | − |
| 1 | 15 | 3.750 | − |
| 1 | 14 | 3.784 | + |
| 1 | 20 | 4.545 | + |
| 2 | 19 | 3.518 | − |
| 2 | 31 | 5.000 | − |
| 2.5–3 | 24 | 3.750 | + |
| 3.5–4 | 14 | 2.258 | − |
| 4 | 93 | 1.390 | − |
| 4 | 17 | 3.696 | − |
| 5 | 19 | 2.714 | − |
| 8 | 17 | 7.083 | − |
| 8 | 14 | 3.500 | − |
| 11 | 8 | 3.077 | − |
| 16 | 19 | 4.750 | − |
| 24–28 | 177 | 1.264 | − |
| * within 24 | 194 | 1.492 | − |
| * 24–48 | 217 | 1.447 | − |

TABLE III

*Functional longevity of* N. obsoletus *hatching substance. Plus represents release of plug from capsule neck; minus represents plug remaining firm in capsule neck.*

| Number larvae hatched | Number larvae per μl | Hours between removal of hatching water and addition of plug | Outcome |
|---|---|---|---|
| 45 | 6.618 | 0 | + |
| 49 | 7.000 | 0 | + |
| 25 | 4.630 | 0 | + |
| 38 | 4.130 | 0 | + |
| 34 | 3.840 | 1.50 | + |
| 41 | 4.254 | 2.25 | − |
| 44 | 4.889 | 2.50 | + |
| 51 | 6.892 | 3.0 | − |
| 47 | 5.014 | 3.0 | − |

The average speed of veligers within the capsule when only a few remained was 1.8 mm per minute ($N = 11$ measurements on 8 individuals). Using this value of $\bar{v}$, the equation predicts that 98% of the veligers will leave the capsule within only 55 minutes. Both of the capsules actually monitored, containing 45 and 51 individuals, lost 98% of their veligers within 45 to 50 minutes after hatching commenced, and the shape of the escape curve is similar to that predicted for a chance escape (Fig. 2). Thus, movements within the capsule are probably undirected. The observed rate of escape is initially in better agreement with that predicted for a slower swimming speed instead of the speed actually determined (Fig. 2). This is perhaps attributable to a decrease in the viscosity of the intracapsular fluid during hatching, since the fluid is soluble in seawater (Costello and Henley, 1971).

## DISCUSSION

The mean number of developing eggs enclosed by a single capsule is highly variable between species, even within the same genus. *Nassarius vibex* capsules,

TABLE IV

*Species specificity of hatching substance produced by veligers of* N. obsoletus *and* N. trivittatus. *Plus represents release of plug from capsule neck; minus represents plug remaining firm in capsule neck.*

| Species | Number embryos | Number embryos per μl | Outcome | |
|---|---|---|---|---|
| | | | N. obsoletus | N. trivittatus |
| *N. obsoletus* | 291 | 1.455 | + | − |
| *N. obsoletus* | 264 | 1.600 | + | − |
| *N. obsoletus* | 19 | 3.800 | + | − |
| *N. obsoletus* | 23 | 4.600 | + | − |
| *N. trivittatus* | 208 | 3.480 | − | + |
| *N. trivittatus* | 224 | 1.600 | − | + |

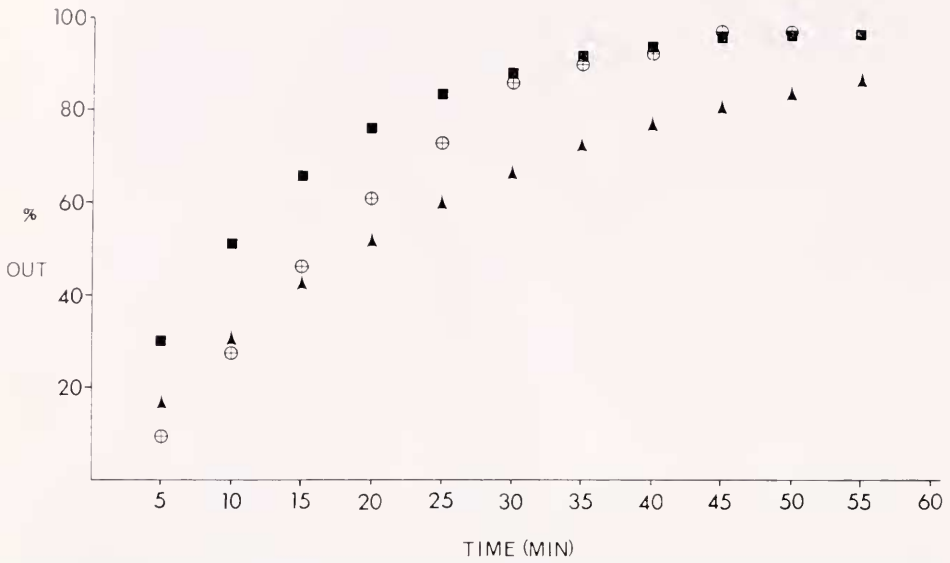


FIGURE 2. The observed and predicted rates of escape of veligers from egg capsules of *N. obsoletus*. Squares trace the predicted rate of escape based on the average swimming speed measured, 1.8 mm per minute. Triangles trace the predicted rate of escape based on a swimming speed of 1.0 mm per minute. Circles trace the average observed rate of escape from two capsules.

for example, generally hold 20 eggs (Scheltema, 1962), *N. pygmaeus* capsules hold 40 to 50 eggs (Lebour, 1937), and *N. obsoletus* capsules hold as many as 300 eggs (Costello and Henley, 1971), although Scheltema (1962) gives a range of 40 to 150. If removal of the capsule operculum is generally a chemical process, one might expect the parcelling of eggs into capsules to be related to the minimum number of individuals required to produce sufficient hatching substance to degrade the plug. However, the actual densities of embryos in *N. obsoletus* capsules are over one hundred times the minimum density required for successful hatching (Table V). For these capsule volumes (Table V), a single individual should be able to remove the operculum and escape.

Hancock (1956) and Kostitzine (1940) implied that the loosening of the operculum is a gradual process in *Urosalpinx cinerea* and *Purpura* (= *Nucella*)

TABLE V

*Embryos per μl in egg capsules of* N. obsoletus.

| Capsule height mm | Number embryos contained | Approximate capsule volume (μl) | Approximate number embryos per μl |
|---|---|---|---|
| 1.25 | 47 | 0.6 | 78 |
| 1.4 | 51 | 0.6 | 85 |
| 1.8 | 152 | 1.8 | 84 |
| 1.3 | 17 | 0.5 | 34 |

*lapillus* capsules, respectively. The only detailed, relevant experimental work done on marine invertebrate hatching is that investigating the dissolution of sea urchin fertilization membranes prior to gastrulation. As in *N. obsoletus,* the substance produced by embryos of the urchin *Strongylocentrotus pulcherrimus* is apparently produced in a short pulse. There is evidence of hatching substance production between 11 and 14 hours after fertilization, but not before or after this period (Sugawara, 1943).

In contrast to the hatching substance produced by *N. obsoletus,* that produced by the urchin *Arbacia punctulata* is said to remain active for several weeks in sea water, although the conditions of storage are not given (Kopac, 1941). Functional longevity of the hatching substance has not been specificially considered for other species studied, although the substance produced by the urchins *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina* was concentrated for experiments by evaporating the hatching water for 3 to 10 hours at room temperature, after which time it was quite potent (Yasumasu, 1960).

The species specificity of the hatching substance produced by the two *Nassarius* species in my study contrasts sharply with the known action of other marine invertebrate hatching substances. Those produced by the urchins *Strongylocentrotus purpuratus* and *S. franciscanus* will dissolve the fertilization membranes of either species (Barrett and Angelo, 1969). In the Ascidiacea, embryos also dissolve away their fertilization membranes. There is no evidence of species specificity of the hatching substance in the five species studied. In fact, the substance produced by *Ascidia conchilega* acts more quickly on the chorion of *Phallusia mammillata* eggs than on the chorion of its own eggs (Berrill, 1929)!

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## Summary

1. The loosening of the egg capsule plug prior to escape of the veligers is shown to be chemically mediated in *Nassarius obsoletus* and *N. trivittatus.*

2. The hatching substance is not produced continuously during development, but rather in a short pulse beginning just prior to hatching and ending within 4 hours of escaping from the capsule, for *N. obsoletus.*

3. The hatching substance produced by the embryos of one species is effective only on the capsule plugs of that species, for the two species studied.

4. The substance is functionally short-lived, at room temperature in sea water, losing its potency within three hours after its secretion by *N. obsoletus.*

5. The observed rate at which *N. obsoletus* veligers leave their egg capsules is shown to be in close agreement with the rate predicted from an equation assuming random movements of individuals within the capsules.

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